Emirates Journal of Food and Agriculture. 2023. 35(12): 1-9
doi: 10.9755/ejfa.2023.3184
http://www.ejfa.me/

RESEARCH ARTICLE

Phenolic content and antioxidant activity in *Lentinula edodes* grown on eucalyptus biomass

María E. Jimenez*, Helen E. Berrus, Joselyn D. Segarra, Oscar L. Peralta, Mercy P. Pólit, Lisbeth C. Campuzano, Raisa A. Carpio, María P. Fondevila, María C. Villacres*

Universidad de Guayaquil, Facultad de Ciencias Químicas, Guayaquil, Ecuador

ABSTRACT

Objective: To determine whether *Lentinula edodes* (*L edodes*) cultured on eucalyptus chips in the Ecuadorian highland generates antioxidant metabolites and anti-inflammatory effects. **Methods:** Total phenolic content was determined by the Folin-Ciocalteu reaction, the *in vitro* antioxidant activity of *L edodes* extracts was evaluated by the DPPH method and the *in vivo* anti-inflammatory activity was studied in mice. The chemical composition was studied by phytochemical screening and gas chromatography/mass spectrometry (GC/MS). **Results:**Total phenols were higher in extracts with increasingly high-water content. The antioxidant activity was robust and significantly strong in these extracts, suggesting the active metabolites are water-soluble. The anti-inflammation activity was significant in aqueous extracts only. Phytochemical screening indicated an overall similar composition to the literature reported earlier. GC/MS detected galactitol, trehalose, xylitol, phosphoric acid and octadecanoic acid among the most abundant metabolites. **Conclusions:** Cultivation on eucalyptus biomass at the Ecuadorian highlands retains the overall chemical composition, the phenolic content, antioxidant levels, and the *in vivo* anti-inflammatory activity of *L edodes*. The 11.3% content of trehalose observed is interesting for its capacity to control cellular stress damage.

Keywords: Inflammation; Antioxidants; Phenols; Shiitake; Lentinula; Phytotherapeutics

INTRODUCTION

Deficiencies in macro and micronutrients are common in unhealthy diets, which is increasingly recognized as linked to a broad range of human health maladies. Such deficiencies have been associated with the frequency of infectious diseases in developing countries (Bhutta and Salam, 2012). Moreover, chronic, non-communicable inflammation-associated diseases are currently the most common burden in the world health (World Health Organization, 2023). Among them are neurodegenerative diseases, type 2 diabetes, atherosclerosis, vascular diseases, arthritis-related diseases, obesity, metabolic syndromes and even cancer, accounting for high health care costs, not only because of the cost of treatments, but also of the economic impact of workhours lost to disease (Rogero and Calder, 2018; Center for Disease Control, 2020, Zagoskina et al 2023).

Inflammation has been implicated as a common factor in all these chronic disorders (Stylianou E, 2019; recently reviewed in Chaudhary MR et al, 2023); in consequence, current research efforts are focused on developing and improving treatments with anti-inflammatory compounds (Krawczyk M. et al, 2023; Tong Z et al., 2023). Accessible and low-cost nutritional foods are a natural source of micronutrients, some of which display beneficial anti-inflammatory properties (Gombart et al., 2020; Zhou T et al., 2023). A current view of mechanisms leading to inflammation is cellular damage induced by overproduction of reactive oxygen (ROS) and nitrogen species (Islam MT, 2017). The reactive species-mediated damage includes changes in electron transport, alterations in cellular energy homeostasis and general mitochondrial failure (Islam MT, 2017). Following this line of research, compounds able to limit ROS-mediated damage are a promising approach for inflammation control.

Interesting research advances report improved immune function following control of oxidative stress after administration of natural compounds (Tan et al, 2018; Ricordi et al, 2015). A well-investigated case is the effect of phenolic compounds in controlling risk factors

***Corresponding authors:**
María C. Villacres, Facultad de Ciencias Químicas, Guayaquil, Ecuador. **E-mail:** maría.villacresc@ug.edu.ec;
María E. Jiménez, Facultad de Ciencias Químicas, Guayaquil, Ecuador. **E-mail:** maria.jimenezhe@ug.edu.ec

Received: 12 July 2023; **Accepted:** 11 November 2023

associated with cardiovascular disease (Lutz et al, 2019). Another intensely studied application is the use of phenolic compounds in several models of cancer (Niedzwiecki et al, 2016). Mushrooms have attracted attention for their notorious antioxidant effects in controlling reactive species among numerous natural products that have been investigated (Sanchez, 2017; Muszyńska et al, 2018; reviewed in Łysakowska P, 2023). *Lentinula edodes* (*L edodes*) is a macro fungus of ancestral application as nutraceutical in Asian cultures, which has been confirmed by more recent studies (Gaitan-Hernandez et al, 2019). The species is at present poorly known in Ecuador, although its nutritive and potential anti-inflammatory properties would be a welcome addition to the habitual diets.

Recent industrial developments have introduced local production of *L edodes* in the Ecuadorian highlands. Several studies elsewhere have reported variation in the composition de *L edodes* reflecting climatic changes and growth conditions (Pedri et al, 2015; Brienzo et al, 2007). Some of these variations may result in changes among secondary metabolites, as already reported for several enzymes (Pedri et al, 2015; Silva, 2008). It is unknown at present what are the effects of local environmental variables, including weather and substrate content, on the composition and biological activities of *L edodes* grown on eucalyptus biomass (Bruhn and Mihail, 2009; Lo et al, 2007; Brauer et al, 2002). This study was designed to verify: i) the anti-inflammatory properties in the locally produced *L edodes*, ii) the general chemical composition including the phenolic content.

MATERIALS AND METHODS

Sample Preparation

Fresh *L edodes* material was obtained from an organic farm in Tumbaco, a rural location 20 km north of Quito, in February 2019 during the rainy season in the Ecuadorian highlands. A sample of 150 units of *L edodes* were selected for uniformity in size and weight according to the Standard Norm 105E. The sample was dried in a laboratory oven and pulverized.

Physical-chemical analysis

Portions of the master sample batch were used for determination of total humidity, total ash content, water-soluble ashes and acid-soluble ashes applying standard laboratory methods (Miranda and Cuellar, 2012).

Extract preparation and phytochemical screening

Extracts were prepared by successive extraction with solvents of increasing polarity: ethylic ether, ethanol, and water. Briefly, 30 g dry and pulverized *L edodes* material was resuspended in 90-150 ml ethylic ether for 48 h at 25ºC. The solid phase after the first extraction was treated with 3x its weight of ethanol for 48 h. The solid phase after the ethanolic extraction was added 3x its weight of sterile distilled water for 48 h. The ether extract was tested for alkaloids, lactones & coumarins, triterpenes and oil content (Supplemental Materials-Table S1). Catechins, resins, reducing sugars, lactones, triterpenes, saponins, phenolic compounds, quinones, flavonoids and amino acids were assayed in the ethanolic extract. Alkaloids, reducing sugars, tannins, saponins and mucilages were assayed in the aqueous extract. Detailed procedures for the phytochemical screening were done as published earlier (Manzano et al, 2017).

Sample silanization

2 mg of dry *L edodes* were derivatized with 120 µl of BSTFA (bis [trimetylsilyl]-trifluorocetamide, Sigma-Aldrich) and maintained at 80ºC for 2 h as described before (Merchant et al, 2010).

Analysis by gas chromatography/mass spectrometry

The silanized samples were analyzed by gas chromatography/mass spectrometry (GC/MS) as reported before (Cevallos-Cevallos and Danyluk, 2011). The details on instrument preparation, analysis and mass spectra identification were as published elsewhere (Manzano, et al., 2017).

Total polyphenols

Total content of polyphenols was determined by the Folin-Ciocalteu method using gallic acid (Sigma Aldrich) as a positive control. Results were expressed as mg gallic acid equivalent (GAE)/100 g sample (Singleton and Rossi, 1965).

In vitro analysis of antioxidant activity

Previous studies highlighted the advantage of methanol to extract secondary metabolites from vegetal species, due to its small molecular size and the easiness to remove it for downstream applications. The antioxidant activity of methanolic extracts was evaluated by the DPPH method as described earlier (Klaus et al, 2013). Briefly, 1,1-diphenyl-2-picrylhydrazyl (DPPH*, 7.9 mg in 100 ml methanol) was used as stock reagent. For each assay reaction, 2.9 ml of DPPH sol were mixed with 0.1 ml sample extract. Absorbance was determined at 517 nm after 30 min incubation at 25ºC in the dark. Trolox (6-hidroxi-2, 5, 7, 8-tetrametilcroman-2-carboxílic acid, Sigma-Aldrich) was used for the preparation of quantification standards (0.13-0.79 µmol Eq Trolox/ml). Inhibition of free radical by DPPH was calculated by the following equation:

$$\text{DPPH scavenging effect (\%)} = [(A_0 - A_1/A_0) \times 100]$$

Where A_0 = absorbance of the control reaction, A_1 = absorbance of the sample reaction.

The IC_{50} values were calculated by linear regression analysis from a graph of scavenging activity plotted against concentration.

In vivo assay for inflammation control

A topical model applying xylene was used to induce chemical inflammation for evaluating the anti-inflammatory capacity of aqueous and ethanolic *L edodes* extracts, as described before (Oh et al, 2018; Hosseinzadeh, 2002). Briefly, 8 g of dry sample were dissolved in 30 ml sterile water and maintained for 2 h at room temperature before filtration. For the ethanolic extract, 8 g sample were mixed with 30 ml ethanol and maintained for 24 h before filtration; the liquid phase was dried to remove the solvent. The extracts were reconstituted in 20 ml sterile water before administration to mice. CD1 mice at 22 g ± 2 g weight were housed in controlled laboratory conditions (22 ± 3°C, relative humidity 50-55%, 12 h light/dark cycles) with free access to water and standard animal food. Animals were restricted on food for the last 12 h before assays. Bio models were weighted and assigned to groups of homogeneous average weight with 5 animals each. The ethanolic and the aqueous extract were administered at 500 mg/kg or 1000 mg/kg. Dexamethasone (Caplin Point Laboratories, 10 mg/kg) was used as positive control of anti-inflammation. A negative control group received sterile water only. Treatments were orally administered at a volume of 400 µl/mouse. Edema was induced on the right ear of each mouse 30 minutes after oral administration of dexamethasone or *E. edodes* extracts by topical xylene application (40 µl xylene on both internal and external sides of the right ear, Sigma Aldrich). The left ear was left untreated as an internal control of inflammation in each mouse. Xylene-induced edema was determined by the weight difference between the left and right ears 30 min after xylene application. Animal management was performed according to the guidelines of the European Union for handling of experimental animals (European Commission, 2010).

Statistical Analysis

Data from the GC/MS chromatograms were analyzed using the INFOSTAT software. Data from chemical assays and bioassays were analyzed applying the statistical software capability of Excel, Microsoft package. Values at $p<0.05$ in two-tailed pair-wise comparisons were considered statistically significant.

RESULTS

Physical chemical analysis

The physical chemical analysis indicated a residual humidity of 7.48 ± 0.37 % in the pulverized sample of *L edodes*. Analysis of the ash content indicated 6.12 ± 0.41 % for water-soluble ashes, 5.66 ± 0.04 % for acid-soluble ashes and 7.30 ± 0.31 % for total ashes (Table 1).

Phytochemical screening

Testing for phytochemical screening was performed in parallel, in ether-based, ethanol-based, and water-based extracts (Table 2). Both the ether and ethanolic extracts gave strong positive reactions to alkaloids by the Dragendorff and the Wagner assays. The Mayer assay gave weak positive reactions for the ethanolic and aqueous extracts. Strong positive reactions were observed for amino acids in the ethanolic extract and for oils in the ether extract. Flavonoids and phenols gave weak positive reactions in the ethanolic and aqueous extracts. The reaction for resins was weak positive in the ethanolic extract, whereas triterpenes and steroids were weakly positive in the ether and ethanol extracts but negative in the aqueous extract. The assays for catechins, anthocyanidins, lactones/coumarins, quinones, saponins and reducing sugars were negative.

Chromatographic analyses

The GM/MS analysis of dehydrated and pulverized *L edodes* was performed using a silanized sample. Overall, 55 compounds were detected in a total run of 49 min. The most abundant compounds detected were: galactitol (44.43 ± 0.63 % AUC), trehalose (11.30 ± 1.57 % AUC), xylitol (9.25 ± 0.13 % AUC), phosphoric acid (3.76 ± 0.16 % AUC), octadecanoic acid (2.28 ± 0.09 % AUC), 9,12-octadecadienoic acid (1.76 ± 0.04 % AUC) and hexadecenoic acid (1.21 ± 0.09 % AUC) (Table 3 and Fig. 1). All other peaks detected represented concentrations

Table 1: Characteristics of residual humidity, total ashes, water-soluble ashes, and acid-soluble ashes in the dried and pulverized sample of *L edodes*.

Variable	Average (%)	SD
Residual humidity	7.48	± 0.37
Total ashes	7.30	± 0.31
Water-soluble ashes	6.12	± 0.41
Acid-soluble ashes	5.66	± 0.04

Table 2: Qualitative results of the phytochemical analyses on *L edodes* extracts

Chemical structure	Ether	Ethanol	Aqueous
Alkaloids-Dragerndorf	+*	+++	+
Alkaloids-Wagner	+++	+++	++
Alkaloids-Mayer	-	+	+
Amino acids	n/a	+++	n/a
Flavonoids	n/a	+	+
Mucilage	-	-	-
Oils	+++	n/a	n/a
Phenols & tannins	n/a	+	+
Resins	n/a	+	n/a
Triterpenes & steroids	+	+	-

*+Weak positive; ++clear positive reaction; +++very positive reaction; - negative reaction; n/a not applicable

below 1 % AUC (Supplemental Table S2). Among detected compounds, 10 were amino acids, 9 were sugars, 6 were short chain fatty acids (succinic acid, glyceric acid, fumaric acid, maleic acid, malic acid, 2-hydroxyglutaric acid), 4 were medium chain fatty acids (dodecanoic acid, 3-deoxy-D-arabinohexonic acid 1,4-lactone, azelaic acid, gluconic acid), 7 were long chain fatty acids (tetradecanoic acid, hexadecanoic acid, heptadecanoic acid, 9,12-octadecadienoic acid, oleic acid, 11-cis-octadecenoic acid, octadecanoic acid). There were also 2 ergosterols: ergosta-7-en-3-beta-ol and ergosta-7,22-dien-3-beta-ol and 2 nucleosides: uridine and adenosine.

Total Polyphenol content

Analysis of polyphenol content gave higher values for the combinations with higher water ratio: 61.9, 72.3 and 77.3 mg GAE/100 g for the 50:50, 70:30 and 90:10 respectively (Fig. 2). The results, run in triplicate, were statistically significant ($p < 0.001$).

Antioxidant activity *in vitro*

The antioxidant activity of methanolic extracts of *L edodes* was evaluated by the DPPH method. The extracts were adjusted at three aqueous combinations: 50:50, 70:30 and 90:10, and at 33, 50 and 100 mg/ml concentration. At all three concentrations, the 90:10 combination gave the highest DPPH reducing values (321-331 µmol TE/g). The 50:50 combination gave increasing DPPH reducing values as the concentration augmented (72, 106 and 221 µmol TE/g at 33, 50 and 100 mg/ml respectively). Similarly, the combination 70:30 gave DPPH reducing values according to the concentration (120, 138 and 221 µmol TE/g at 33, 50 and 100 mg/ml respectively). For each concentration, the determinations were statistically significant between the different combinations, except for the 50:50 and 70:30 ratios at the 100 mg/ml concentration (Fig. 3a).

Values from the DPPH reduction assay were plotted against concentration, and IC_{50} values were determined by linear regression analysis. The IC_{50} correlated inversely with the solvent concentration. Thus, the extract prepared with the water: methanol solvent ratio 90:10 was the most potent (28.8 mg/mL), whereas the extract 70:30 and the extract 50:50 required higher concentration to reach the IC_{50} (79.1 and 92.5 mg/mL respectively, Fig. 3b).

Effect of E edodes extracts on inflammation

Local inflammation was induced in groups of mice by xylene application on their right ear. Orally administered aqueous and ethanolic *L edodes* extracts were evaluated for their anti-inflammatory activity at 497.5 mg/mouse (500 µl) or 995 mg/mouse (1000 µl). The difference in weight between the treated (right) ear and the untreated left ear of each mouse after 2 h of treatment was determined. The aqueous *L edodes* extract at the 500 µl dose was the most efficient in local anti-inflammatory effect (6.0 mg difference compared to 2.3 mg difference for the dexamethasone positive control). The higher *L edodes* aqueous extract and the two ethanolic extract did not generated any substantial inflammation control (Fig. 4).

DISCUSSION

Assays for the antioxidant activity in vitro in the *L edodes* extracts detected a robust signal in the DPPH scavenging assay. This activity was stronger in the preparations with higher water content, suggesting that the relevant metabolites were water-soluble. The observation was verified by the IC_{50} calculation, where the preparations with higher water content relative to the solvent methanol (90:10) gave the lowest IC_{50} values. The total phenolic content of the preparations followed a similar relation with the water content, with higher mg GAE values in combination with more aqueous content. Overall, our results agree with previous studies indicating substantial antioxidant activity in *L edodes* extracts (Poniedzialek et al, 2019); Chowdhury et al, 2015; Kozarski et al, 2012).

The *in vivo* assay to evaluate anti-inflammatory activity detected an interesting activity with 497.5 mg (500 µl) dose of aqueous extract, but not at a higher dose or in ethanolic extracts of either low or high dose. Data from the scavenging DPPH assay as well as the phenolic content correlate well with results from the anti-inflammatory activity *in vivo* using the aqueous extract. Although our values were less pronounced than the positive drug control, they agree with previous studies reporting control of inflammation by *L edodes* in other *in vivo* models. Chemically induced colitis in mice was alleviated by *L edodes* glucans, likely associated with reduced expression of IL8 mRNA

Table 3: Main compound peaks detected by GC/MS in samples of *L edodes*

Peak	Compound	Formula	Retention time (min)	Concentration (% AUC±SD)
1	Phosphoric acid	H_3PO_4	12.780	3.76±0.16
2	Xylitol	$C_5H_{12}O_5$	23.803	9.25±0.13
3	Galactitol	$C_6H_{14}O_6$	28,235	44.43±0.63
4	Hexadecenoic acid	$C_{16}H_{32}O_2$	30.313	1.21±0.09
5	9,12-octadecadienoic acid	$C_{18}H_{32}O_2$	33.251	1.76±0.04
6	Octadecanoic acid	$C_{18}H_{36}O_2$	33.880	2.28±0.09
7	Trehalose	$C_{12}H_{22}O_{11}$	41.672	11.30±1.57

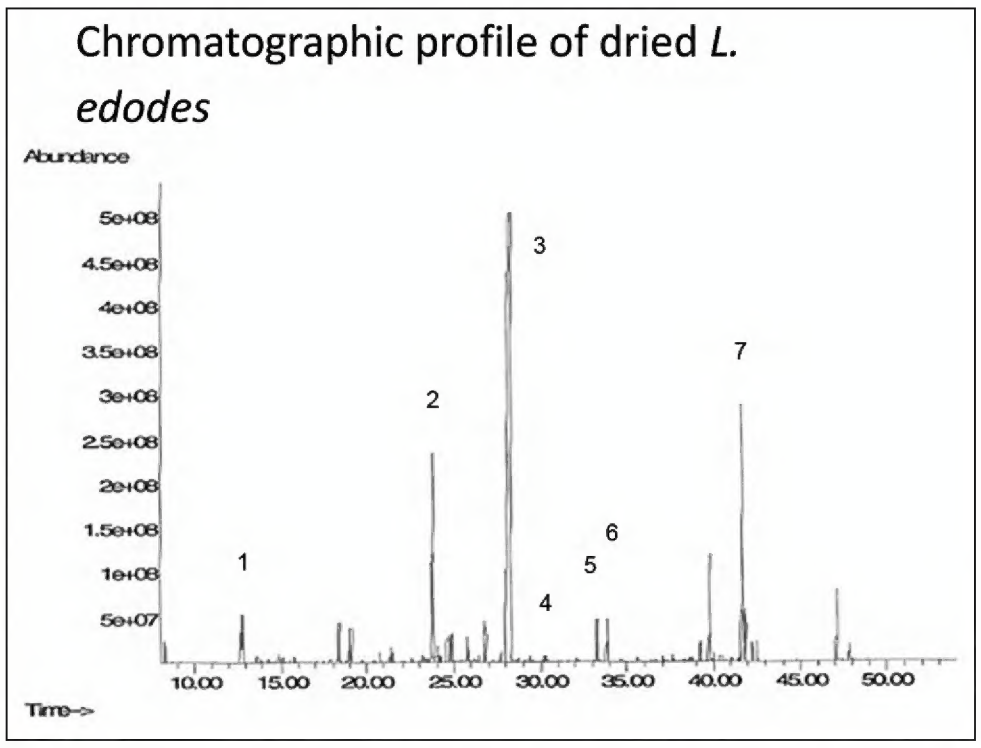

Fig 1. Gas chromatograph of the dehydrated pulverized sample of L edodes. The most abundant components detected were phosphoric acid (1), xylitol (2), galactitol (3), hexadecenoic acid (4), 9,12-octadecadienoic acid (5), octadecanoic acid (6) and trehalose (7).

Fig 2. Total phenolic content in L edodes extracts at three ratios water: methanol. Results are expressed as mg GAE/100 g (mg Gallic Acid Equivalent/100 g) extract. All comparisons were statistically significant (** p< 0.001).

in intestinal tissues (Shi et al, 2016; Nishitani et al, 2013). A model of LPS-mediated lung injury was associated with limited cytokine secretion (TNFα, IL6 and IL1β) and reduced oxidative state in mice (Ren et al, 2018). Some previous studies identified the effect of *L edodes* glucans on the MAPK pathway while others reported reduced NO production as a mechanism of protection (Shi et al, 2016; Liu et al, 2016; Gunawardena et al, 2014; Pacheco-Sanchez et al, 2006). Our study aimed to verify an anti-inflammatory effect following ingestion of *L edodes* extract, as the oral is the most common route of use in the local population. The observed inflammation reducing effect appears to be mediated by water-soluble components, of which the lentinan may be the most likely mediator. Regarding inflammation, our observations are also in agreement with recent studies (Morales et al, 2020; Kupcova et al, 2018).

The analysis by GC/MS detected a significant level of sugars, including galactitol, trehalose and xylitol, accounting for almost 70% of all compounds detected. The abundance of carbohydrates has been reported before, and recent studies by GC/MS confirm the overall dominance of sugars/glycols, organic acids, and amino acids (Zhao et al, 2019; Reis et al, 2012). Our analysis detected a high content of the hexose galactitol. An earlier and detailed study of methylated and acetylated exopolysaccharides in *L edodes* preparations indicated a significant content of glucose, mannose, xylose, galactose, fucose, rhamnose and arabinose (Lo et al, 2007). Method differences during sample preparation prior to GC/MS analysis may explain the dissimilar results.

Among carbohydrates, trehalose is recognized for its role not only as an efficient energy source but also in protecting cells from stress damage (Benaroudj et al, 2001). Interesting in our data, trehalose had a significant level of 11.3 % ACU, and continuation studies may focus on its potential role in a human diet. Another significant observation was the presence of fatty acids: octadecanoic, 9,12-octadecadienoic

Fig 3. (a) Antioxidant activity in L edodes extracts. Results are expressed as µmol TE/g (Trolox Equivalent/g) determined in water: methanol extracts, containing 33, 50 or 100 mg/ml of L edodes pulverized material. All comparisons were statistically significant (** p < 0.001) except in the100 mg/ml group for the ratios 50:50 and 70:30. (b) IC50 values for L edodes extracts in three combinations of water: methanol. The combination 90:10 gave the lowest value for IC50 (28.8 µg/ml), compared to the ratios 50:50 (92.5 µg/ml) and 70:30 (79.1 µg/ml).

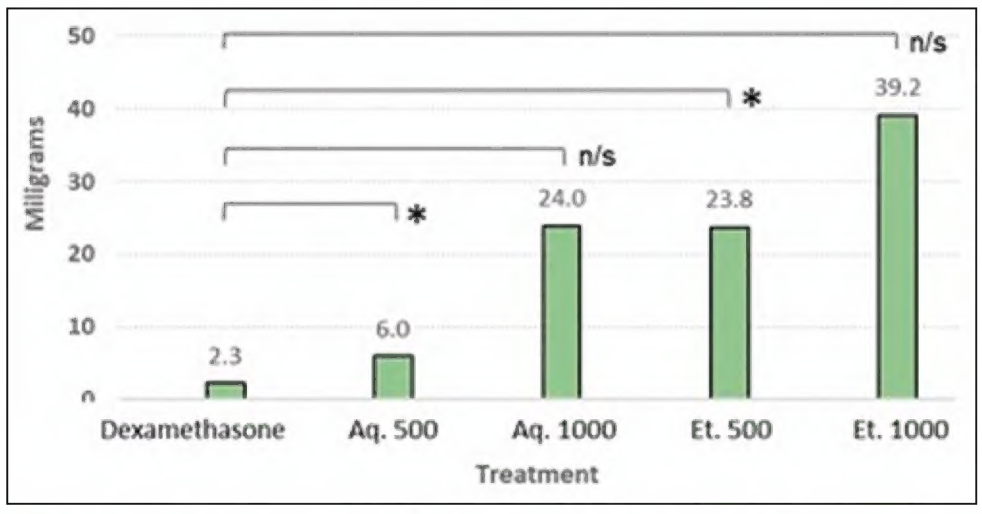

Fig 4. Anti-inflammatory effect of L. edodes extracts in chemically induced edema in mice. The results show the difference in weight between treated and non-treated ears of groups of mice with xylene-induced inflammation. Mice were treated with dexamethasone (positive control of treatment), aqueous L edodes extracts (500 or 1000 µl) or ethanolic L edodes extracts (500 or 1000 µl). *, $p< 0.05$; n/s, no statistical significance.

and hexadecanoic acids. This type of lipid exerts interesting biological properties in the control of inflammation and associated pathologies (DiNicolantonio and O'Keefe, 2017; Astudillo et al, 2018). All other lipid components were detected at concentrations below 1%. Among those were several fatty acids (dodecanoic acid, heptadecanoic acid, tetra decanoic acid, oleic acid, and 11-cis-octadecenoic acid).

The polysaccharide lentinan is one of the best studied metabolites in *L. edodes*. Several biological effects, including anti-inflammatory and anticancer properties, have been investigated in different models (Gaitan-Hernandez et al, 2019; Xu et al, 2014; Bisen et al, 2010). Our results confirm the substantial levels of antioxidant components in *L. edodes* grown in the Ecuadorian highlands, and their anti-inflammation effect in laboratory animals. Further studies may clarify whether this effect is mediated solely by lentinan, and the role of fatty acids. Previous studies detected significant effects of growing conditions in the chemical composition of *L. edodes*, thus it was relevant to verify the effect of the local environmental conditions on the fungal components and potential anti-inflammatory effects (Reis et al, 2012; Manzi et al, 1999).

CONCLUSIONS

L. edodes grown on eucalyptus biomass presents significant antioxidant activity *in vitro*. Thus, the local environment and substrate do not affect the content of antioxidant components. The significance of this activity was confirmed by the anti-inflammatory activity *in vivo*, both in water-soluble extracts. Finally, the data validate that the phenolic content and the chemical composition in the local *L. edodes* is in general agreement with the reported literature.

ACKNOWLEDGEMENTS

Funding for the study was provided by the Dirección de Investigación de la Universidad de Guayaquil.

Author disclosure statement

No competing financial interests exist.

Authors contribution

Helen E. Berrus and Joselyn D. Segarra performed the physical-chemical analyses, the experiments for phytochemical screening and the gas chromatography/mass spectrometry analysis. Oscar L. Peralta and Mercy P. Pólit did the quantifications of polyphenols and the analysis of antioxidant activity. Lisbeth C. Campuzano and Raisa A. Carpio carried out the evaluation of anti-inflammatory activity *in vivo*. María E. Jimenez, María P. Fondevila and María C. Villacres designed the study, led the experiments, and wrote the report. All authors participated in the statistical analyses.

REFERENCES

Astudillo, A. M., C. Meana, C. Guijas, L. Pereira, P. Lebrero, M. A. Balboa and J. Balsinde. 2018. Occurrence and biological activity of palmitoleic acid isomers in phagocytic cells. J Lipid Res. 59: 237-249.

Benaroudj, N., D. H. Lee and A. L. Goldberg. 2001. Trehalose accumulation during cellular stress protects cells and cellular proteins from damage by oxygen radicals. J Biol Chem. 276: 24261-24268.

Bhutta, Z. A. and R. A. Salam. 2012. Global nutrition epidemiology and trends. Ann Nutr Metab. 61 Suppl 1: 19-27.

Bisen, P. S., R. K. Baghel, B. S. Sanodiya, G. S. Thakur and G. B. Prasad. 2010. *Lentinus edodes*: A macrofungus with pharmacological activities. Curr Med Chem. 17: 2419-2430.

Brauer, D., T. Kimmons and M. Phillips. 2002. Effects of management on the yield and high-molecular-weight polysaccharide content of shiitake (*Lentinula edodes*) mushrooms. J Agric Food Chem. 50: 5333-5337.

Brienzo, M., E. M. Silva and A. M. Milagres. 2007. Degradation of eucalypt waste components by *Lentinula edodes* strains detected by chemical and near-infrared spectroscopy methods. Appl Biochem Biotechnol. 141: 37-50.

Bruhn, J. N. and J. D. Mihail. 2009. Forest farming of shiitake mushrooms: Aspects of forced fruiting. Bioresour Technol. 100: 5973-5978.

Centers for Disease Control and Prevention. 2020. About Chronic Diseases. Atlanta, GA: Centers for Disease Control and Prevention, U.S. Department of Health and Human Services. Availabe from: https://www.cdc.gov/chronicdisease/index.htm

Cevallos-Cevallos, J. M., M. D. Danyluk and J. I. Reyes-De-Corcuera. 2011. GC-MS based metabolomics for rapid simultaneous detection of *Escherichia coli* O157:H7, *Salmonella* Typhimurium, *Salmonella* Muenchen, and Salmonella Hartford in ground beef and chicken. J Food Sci. 76: M238-M246.

Chaudhary, M. R., S. Chaudhary, Y. Sharma, T. A. Singh, A. K. Mishra, S. Sharma, M. M. Mehdi. 2023. Aging, oxidative stress and

degenerative diseases: mechanisms, complications and emerging therapeutic strategies. Biogerontology, 24: 609-662.

Chowdhury, M., K. Kubra and R. Ahmed. 2015. Screening of antimicrobial, antioxidant properties and bioactive compounds of some edible mushrooms cultivated in Bangladesh. Ann Clin Microbiol Antimicrob. 14: 8.

DiNicolantonio, J. J. and J. H. O'Keefe. 2017. Good fats versus bad fats: A comparison of fatty acids in the promotion of insulin resistance, inflammation, and obesity. Mo Med. 114: 303-307.

European Commission. 2010. Legislation for the Protection of Animals Used for Scientific Purposes. Available from: https://ec.europa.eu/environment/chemicals/lab_animals/legislation_en.htm

Gaitán-Hernández, R., D. López-Peña, M. Esqueda and A. Gutiérrez. 2019. Review of bioactive molecules production, biomass, and basidiomata of shiitake culinary-medicinal mushrooms, *lentinus edodes* (*Agaricomycetes*). Int J Med Mushrooms. 21: 841-850.

Gombart, A. F., A. Pierre and S. Maggini. 2020. A review of micronutrients and the immune system-working in harmony to reduce the risk of infection. Nutrients, 12: 236-277.

Gunawardena, D., L. Bennett, K. Shanmugam, K. King, R. Williams, D. Zabaras, R. Head, L. Ooi, E. Gyengesi and G. Munch. 2014. Anti-inflammatory effects of five commercially available mushroom species determined in lipopolysaccharide and interferon-γ activated murine macrophages. Food Chem. 148: 92-96.

Hosseinzadeh, H. and H. M. Younesi. 2002. Antinociceptive and anti-inflammatory effects of *Crocus sativus L.* stigma and petal extracts in mice. BMC Pharmacol. 2: 7-15.

Islam, M. T. 2017. Oxidative stress and mitochondrial dysfunction-linked neurodegenerative disorders. Neurol Res. 39: 73-82.

Klaus, A., M. Kozarski, M. Niksic, D. Jakovljevic, N. Todorovic, I. Stefanoska and L. J. L. D. Van Griensven. 2013. The edible mushroom *Laetiporus sulphureus* as potential source of natural antioxidants. Int J Food Sci Nutr. 64: 599-610.

Kozarski, M., A. Klaus, M. Niksi, M. Vrvic, N. Todorovic, L. J. L. D. Van Griensven and D. Jakovljevic. 2012. Antioxidative activities and chemical characterization of polysaccharide extracts from the widely used mushrooms *Ganoderma applanatum*, *Ganoderma lucidum*, *Lentinus edodes* and *Trametes versicolor*. J Food Compos. 26: 144-153.

Krawczyk, M., I. Burzynska-Pedziwiatr, L. A. Wozniak and M. Bukowiecka-Matusiak. 2023. Impact of polyphenols on inflammatory and oxidative stress factors in diabetes mellitus: Nutritional antioxidants and their application in improving antidiabetic therapy. Biomolecules. 13: 1402-1432.

Kupcova, K., I. Stefanova, Z. Plavcova, J. Hosek, P. Hrouzek and R. Kubec. 2018. Antimicrobial, cytotoxic, anti-inflammatory, and antioxidant activity of culinary processed shiitake medicinal mushroom (*Lentinus edodes*, *Agaricomycetes*) and its major sulfur sensory-active compound-lenthionine. Int J Med Mushrooms. 20: 165-175.

Liu, Q., L. Dong, H. Li, J. Yuan, Y. Peng and S. Dai. 2016. Lentinan mitigates therarubicin-induced myelosuppression by activating bone marrow-derived macrophages in an MAPK/NF-κB-dependent manner. Oncol Rep. 36: 315-323.

Lo, T. C., M. W. Kang, B. C. Wang and C. A. Chang. 2007. Glycosyl linkage characteristics and classifications of exo-polysaccharides of some regionally different strains of *Lentinula edodes* by amplified fragment length polymorphism assay and cluster analysis. Anal Chim Acta. 592: 146-153.

Lutz, M., E. Fuentes, F. Ávila, M. Alarcón and I. Palomo. 2019. Roles of phenolic compounds in the reduction of risk factors of cardiovascular diseases. *Molecules*. 24: 366-381.

Łysakowska, P., A. Sobota and A. Wirkijowska. 2023. Medicinal mushrooms: Their bioactive components, nutritional value and application in functional food production-a review. Molecules. 28: 5393-5408.

Manzano, P. I., O. G. Pesantes, G. M. Sarmiento, I. Chóez-Guaranda, Z. C. Burbano, G. M. Duran and M. C. Villacres. 2017. Chemical analysis and normo-glycemic effect of *Opuntia Ficus Indica*. Pharm Online. 1: 19-27.

Manzi, P., L. Gambelli, S. Marconi, V. Vivanti and L. Pizzoferrato. 1999. Nutrients in edible mushrooms: An inter-species comparative study. Food Chem. 65: 477-482.

Merchant, A., A. D. Peuke, C. Keitel, C. Macfarlane, C. R. Warren and M. A. Adams. 2010. Phloem sap and leaf delta 13C, carbohydrates, and amino acid concentrations in *Eucalyptus globulus* change systematically according to flooding and water deficit treatment. J Exp Bot. 61: 1785-1793.

Miranda, M. and A. Cuellar. 2012. Farmacognosia y Química de Productos Naturales. 2nd ed. Editorial Félix Varela, Habana, Cuba, p. 127-130.

Morales, D., R. Rutckeviski, M. Villalva, H. Abreu, C. Soler-Rivas, S. Santoyo, M. Iacomini and F. R. Smiderle. 2020. Isolation and comparison of α-and β-D-glucans from shiitake mushrooms (*Lentinula edodes*) with different biological activities. Carbohydr Polym. 229: 115521-115533.

Muszyńska, B., A. Grzywacz-Kisielewska, K. Kała and J. Gdula-Argasińska. 2018. Anti-inflammatory properties of edible mushrooms: A review. Food Chem. 243: 373-381.

Niedzwiecki, A., M. W. Roomi, T. Kalinovsky and M. Rath. 2016. Anticancer efficacy of polyphenols and their combinations. Nutrients. 8: 552-569.

Nishitani, Y., L. Zhang, M. Yoshida, T. Azuma, K. Kanazawa, T. Hashimoto and M. Mizuno. 2013. Intestinal anti-inflammatory activity of lentinan: Influence on IL-8 and TNFR1 expression in intestinal epithelial cells. PLoS One. 8: e62441-e62452.

Oh, Y. C., Y. H. Jeong, W. K. Cho, Y. H. Hwang and J. Y. Ma. 2018. Inhibitory effects of Dianthi Herba ethanolic extract on inflammatory and nociceptive responses in murine macrophages and mouse models of abdominal writhing and ear edema. J Ethnopharmacol. 211: 375-383.

Pacheco-Sanchez, M., Y. Boutin, P. Angers, A. Gosselin and R. J. Tweddell. 2006. A bioactive (1-->3)-, (1--4)-beta-D-glucan from *Collybia dryophila* and other mushrooms. Mycologia. 98: 180-185.

Pedri, Z. C., L. M. Lozano, K. L. Hermann, C. V. Helm, R. M. Peralta and L. B. Tavares. 2015. Influence of nitrogen sources on the enzymatic activity and grown by *Lentinula edodes* in biomass *Eucalyptus benthamii*. Braz J Biol. 75: 940-947.

Poniedziałek, B., M. Siwulski, A. Wiater, I. Komaniecka, A. Komosa, M. Gasecka, Z. Magdziak, M. Mleczek, P. Niedzielski, J. Proch, M. Ropacka-Lesiak, M. Lesiak, E. Henao and P. Rymski. 2019. The effect of mushroom extracts on human platelet and blood coagulation: *In vitro* screening of eight edible species. Nutrients. 11: 3040-3062.

Reis, F. S., L. Barros, A. Martins and I. C. Ferreira. 2012. Chemical composition and nutritional value of the most widely appreciated cultivated mushrooms: An inter-species comparative study. Food Chem Toxicol. 50: 191-197.

Ren, Z., J. Li, X. Song, J. Zhang, W. Wang, X. Wang, Z. Gao, H. Jing, S. Li and L. Jia. 2018. The regulation of inflammation and oxidative status against lung injury of residue polysaccharides by *Lentinula edodes*. Int J Biol Macromol. 106: 185-192.

Ricordi, C., M. Garcia-Contreras and S. Farnetti. 2015. Diet and inflammation: Possible effects on immunity, chronic diseases,

and life span. J Am Coll Nutr. 34 Suppl 1: 10-13.

Rogero, M. M. and P. C. Calder. 2018. Obesity, inflammation, toll-like receptor 4, and fatty acids. *Nutrients.* 10: 432-451.

Sanchez, C. 2017. Reactive oxygen species and antioxidant properties from mushrooms. Synthetics and Systems Biotechnology. 2: 13-22.

Shi, L., Q. Lin, T. Yang, Y. Nie, X. Li, B. Liu, J. Shen, Y. Liang, Y. Tang and F. Luo. 2016. Oral administration of *Lentinus edodes* β-glucans ameliorates DSS-induced ulcerative colitis in mice via MAPK-Elk-1 and MAPK-PPARγ pathways. Food Funct. 7: 4614-4627.

Silva, E. M., S. F. Martins and A. M. F. Milagres. 2008. Extraction of manganese peroxidase produced by *Lentinula edodes*. Bioresour. Technol. 99: 2471-2475.

Singleton, V. L. and J. A. Rossi. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Viticult. 16: 144-158.

Stylianou, E. 2019. Epigenetics of chronic inflammatory diseases. J Inflamm Res. 12: 1-14.

Tan, B. L., M. E. Norhaizan, W. P. P. Liew and H. Sulaiman Rahman. 2018. Antioxidant and oxidative stress: A mutual interplay in age-related diseases. Front. Pharmacol. 9: 1162-1190.

Tong Z., G. Chu, C. Wan, Q. Wang, J. Yang, Z. Meng, L. Du, J. Yang and H. Ma H. 2023. Multiple metabolites derived from mushrooms and their beneficial effect on Alzheimer's diseases. Nutrients. 15: 2758-2785.

World Health Organization. 2023. World Health Data Platform. Noncommunicable Diseases. Available from: https://www.who.int/news-room/fact-sheets/detail/noncommunicable-diseases

Xu, X., H. Yan, J. Tang, J. Chen and X. Zhang. 2014. Polysaccharides in *Lentinus edodes*: Isolation, structure, immunomodulating activity and future prospective. Crit Rev Food Sci Nutr. 54: 474-487.

Zagoskina, N. V., M. Y. Zubova, T. L. Nechaeva, V. V. Kazantseva, E. A. Goncharuk, V. M. Katanskaya, E. N. Baranova and M. A. Aksenova. 2023. Polyphenols in plants: Structure, biosynthesis, abiotic stress regulation, and practical applications (Review). Int J Mol Sci. 24: 13874-13899.

Zhao, X., M. Chen, Y. Zhao, L. Zha, H. Yang and Y. Wu. 2019. GC/MS-based nontargeted and targeted metabolic profiling identifies changes in the lentinula edodes mycelial metabolome under high-temperature stress. Int J Mol Sci. 20: 2330-2350.

Zhou, T., Q. Li, M. Zhao, Y. Pan and X. Kong. 2023. A review on edible fungi-derived bioactive peptides: Preparation, purification and bioactivities. Int J Med Mushrooms. 25: 1-11.

SUPPLEMENTAL TABLE

Supplemental Table S1: Assays included in the phytochemical analysis of *L edodes* extracts

Chemical Group	Assay
alkaloids	Dragerndorf, Mayer & Wagner
amino acids	ninhydrin
anthocyanidins	
catechins	
flavonoids	Shinoda
foam	saponins
lactones & coumarins	Baljet
mucilage	
oils	Sudan
phenols & tannins	Ferric chloride
quinones	Borntrager
reducing sugars	Fehling
resins	
triterpenes & steroids	Lieberman-Buchard

Table S1: Components of *L edodes* detected by GC/MS analysis

	Time (s)	Name	% AUC	± SE
1	8.299	alanine	0.43	0.13
2	9.251	2-pyrrolidinone	0.06	0.01
3	12.4	urea	0.02	0
4	12.78	phosphoric acid	3.76	0.16
5	12.865	glycerol	0.85	0.04
6	13.848	succinic acid	0.07	0.01
7	14.309	glyceric acid	0.03	0.01
8	14.773	fumaric acid	0.04	0
9	14.864	maleic acid	0.22	0.01
10	15.1	serine	0.17	0.01
11	15.749	threonine	0.17	0.01
12	17.447	2-piperidone	0.05	0.01
13	18.383	malic acid	0.98	0.03
14	18.688	erythrose	0.06	0
15	19.038	pyroglutamic acid	0.96	0.05
16	19.114	aspartic acid	0.09	0
17	20.281	dodecanol	0.07	0.01
18	20.444	2-hydroxyglutaric acid	0.05	0
19	20.756	2,4,6-tri-tert-butylphenol	0.25	0.02
20	21.354	ornithine	0.34	0.01
21	21.47	glutamic acid	0.24	0.01
22	22.185	dodecanoic acid	0.05	0
23	22.543	asparagine	0.1	0.01
24	23.421	lysine	0.12	0.01
25	23.803	xylitol	9.25	0.13
26	24.63	glycerophosphoglycerol	0.71	0.01
27	24.849	glutamine	0.75	0.01
28	24.91	1,4 lactone of 3-deoxy-D-arabinohexonic acid	0.08	0
29	25.103	terepthalic acid	0.02	0
30	25.321	azelaic acid	0.02	0.01
31	25.502	fructofuranose	0.08	0.01
32	25.807	citric acid	0.58	0.01
33	26.411	tetradecanoic acid	0.13	0.01
34	26.8	3-deoxyhexitol	0.94	0.07
35	28.235	dulcitol	44.43	0.63
36	28.282	glucitol	0.16	0.01
37	29.071	galactose	0.04	0
38	29.162	pantothenic acid	0.04	0.03
39	29.357	gluconic acid	0.24	0
40	29.44	allofuranose	0.02	0.01
41	30.313	hexadecanoic acid	1.21	0.09
42	31.018	inositol	0.07	0.01
43	31.895	heptadecanoic acid	0.09	0.01
44	33.136	tryptophan	0.02	0
45	33.251	9,12-octadecadienoic acid	1.76	0.04
46	33.333	oleic acid	0.09	0.02
47	33.441	11-cis-octadecenoic acid	0.03	0.01
48	33.88	octadecanoic acid	2.28	0.09
49	35.558	mannitol	0.13	0.01
50	37.074	uridine	0.16	0
51	37.609	isatine	0.17	0.01
52	39.988	adenosine	0.24	0.01
53	41.672	trehalose	11.3	1.57
54	48.068	ergosta-7,22-dien-3-beta-ol	0.05	0.03
55	48.869	ergosta-7-en-3-beta-ol	0.09	0.01